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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (280 characters max)

EEF1A-2 FOR USE IN THE PROGNOSIS, DIAGNOSIS AND TREATMENT OF CANCER

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* **15** ☐ CD(s), Number

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☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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Respectfully submitted,

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1) Patent Application Transmittal Letter (2 copies);

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Kathleen A. Tyrrell
KATHLEEN A. TYRRELL

EEF1A-2 FOR USE IN THE PROGNOSIS, DIAGNOSIS AND TREATMENT OF CANCER

Field of the Invention

EEF1A2, the gene encoding protein elongation factor EEF1A-2 (eEF-1α2), has now been demonstrated to be amplified in various tumors. Further, as also demonstrated herein, EEF1A-2 has properties of an oncogene in that it enhances focus formation, allows anchorage independent growth and decreases the doubling time of fibroblasts, promotes tumorigenicity in fibroblasts and increases the growth rate of ovarian carcinoma cells xenografted in nude mice. The present invention provides methods and kits for diagnosing and prognosticating cancer via detection of *EEF1A2* and/or EEF1A-2. The present invention also provides methods for treating cancer via inhibition of expression and/or activity of EEF1A-2, screening assays to identify new anticancer agents based upon their ability to inhibit EEF1A-2 expression and/or activity, and compositions comprising an inhibitor of EEF1A-2 expression and/or activity for use in the treatment of various cancers. The methods, kits and compositions of the present invention are particularly useful in the prognosis, diagnosis and treatment of ovarian cancer, as well as breast and colorectal cancer.

Background of the Invention

In the year 2001, 25,000 North American women were expected to be diagnosed with ovarian cancer. Over half of the women diagnosed with ovarian cancer are likely to die of this disease.

Amplification of the 20q13 locus is a marker for late stages of ovarian cancer (Courjal et al. Br. J. Cancer 1996 74:1984-1989) and the presence of four or more copies of 20q13 is associated with a decreased five-year survival after diagnosis (Diebold et al. J. Pathol. 2000 190:564-571). Specifically, a 20-30% fraction of ovarian tumors have an increase in copy number of the 20q13 locus (Courjal et al. Br. J. Cancer 1996 74:1984-1989; Sonoda et al. Genes Chromosomes Cancer 1997 20:320-328; and Diebold et al. J. Pathol. 2000 190:564-571) thus implicating one or more genes at 20q13 in the genesis and progression of ovarian tumors.

Multiple genes map to the 20q13 locus including, but not limited to *ZNF217*, *NABC1* (Collins et al. Proc. Natl Acad. Sci USA 1998 95:8703-8708), *CYP24* (Albertson et al. Nat. Genet. 2000 25:144-146) and *STK15/BTAK* (Bischoff et al. EMBO J. 1998 17:3052-3065). Neither *CYP24*, nor *NABC1* are known to have tumorigenic properties. However, mapping of the breast 20q13 amplicon by CGH (comparative genomic hybridization) suggests that the DNA amplifications center on a ~2Mb region around 20q13.2 and *CYP24* (Albertson et al. Nat. Genet. 2000 25:144-146), the gene for vitamin D24 hydrolase (Walters, M.R. Endocri. Rev. 1992 13:719-764), implicating this gene as the so-called "amplicon driver" for 20q13 in breast cancer. In addition, *ZNF217* has been disclosed as promoting the immortalization of mammary epithelial cells (Nonet et al. Cancer Res. 2001 61:1250-1254) and *STK15* has been disclosed as a transformer (Bischoff et al. EMBO J. 1998 17:3052-3065).

Another gene that maps to the 20q13 locus is *EEF1A2* (Lund et al. Genomics 1996 36:359-361). *EEF1A2* encodes protein elongation factor EEF1A-2 (formerly eEF-1α2). During protein translation, eukaryotic elongation factors (EEF) control the recruitment of amino-acylated tRNA to the ribosome and regulate the translocation of the growing polypeptide from the ribosome A to P sites (Hershey et al. Annu. Rev. Biochem. 1991 60:717-755). Human EEF1A-2 is one of two isoforms of eukaryotic elongation factor 1 alpha (EEF1A-1 and EEF1A-2) that share >90% DNA sequence and amino acid identity. EEF1A proteins bind and hydrolyze GTP and catalyze the association of tRNAs to the ribosome during protein elongation (Hershey et al. Annu. Rev. Biochem. 1991 60:717-755). In addition to their role in protein translation, EEF1A proteins from a variety of sources bind to F-actin (Condeelis, J. Trends Biochem. Sci. 1995 20:169-170; Yang et al. Nature 1990 347:494-496) and depolymerize α-tubulin microtubules (Shina et al. Science 1994 266:282-285). Accordingly, it is believed that these proteins have a role in regulating cytoskeletal organization.

A homozygous deletion of the first intron and promoter of the *EEF1A2*, termed the *wst* allele, occurs in the Wasted mouse, a spontaneous HRS/J variant (Shultz et al. Nature 1982 297:402-404; Chambers et al. Proc. Natl Acad. Sci. USA 1998 95:4463-4468). The deletion prevents EEF1A-2 transcription (Chambers et al. Proc. Natl Acad. Sci. USA 1998 95:4463-4468). EEF1A-2-deficient Wasted mice suffer a B-and T-cell immuno-deficiency and neuromuscular abnormalities (Shultz et al. Nature 1982 297:402-404) and die by 30 days of age of unknown cause. Wasted mice display an increase in lymphocyte apoptosis

relative to EEF1A-2 +/– animals and the possibility that EEF1A-2 may be an inhibitor of apoptosis has been raised (Potter et al. Cell Immunol. 1998 188:111-117).

Summary of the Invention

An aspect of the present invention relates to methods for diagnosing and prognosticating various cancers in a subject comprising measuring *EEF1A2* or EEF1A-2 levels in a biological sample obtained from the subject and comparing the measured *EEF1A2* or EEF1A-2 levels with levels of *EEF1A2* or EEF1A-2 in a control wherein an increase in the measured *EEF1A2* or EEF1A-2 levels as compared to the control is indicative of the subject having cancer.

Another aspect of the present invention relates to kits for detecting *EEF1A2* or EEF1A-2 levels in a biological sample for use in diagnosing and prognosticating cancer in a subject.

Another aspect of the present invention relates to methods for treating various cancers comprising administering to a patient suffering from cancer an inhibitor of EEF1A-2 expression and/or activity.

Yet another aspect of the present invention relates to screening assays to identify new anticancer agents based upon the ability of an agent to inhibit EEF1A-2 expression and/or activity.

Brief Description of the Figures

Figures 1a through 1e show amplification of *EEF1A2* in ovarian tumors detected by FISH. An *EEF1A2*-containing BAC clone and a control 20p11 probe were hybridized to nuclei from human ovarian tumors. In Figures 1a-1c, 8-10 *EEF1A2*-hybridizing loci are visible in each of these three tumors, while only 2-4 copies of 20p11 are visible. In Figure 1d, 2 copies each of *EEF1A2* and 20p11 are visible in this ovarian tumor. Figure 1e is a metaphase spread showing that the *EEF1A2* BAC maps to 20q13.

Figures 2a and 2b show the increased expression of EEF1A-2 mRNA in ovarian tumors and cell lines. As shown in Figure 2a, EEF1A-2 mRNA was readily detected in 3 of 11 ovarian tumor samples but was not detected in normal ovary mRNA (N). By stripping the membrane and re-probing with GAPDH and EEF1A-1, approximately equal loading was shown. Each of the tumors was also tested for *EEF1A2* amplifications and the presence of increased copy number is shown. Figure 2b shows the expression of EEF1A-2

to be detectable in the ovarian cancer cell lines TOV-112D, PA-1, HEY and 2008 but not in the normal ovarian cell line NOV-61 or OV-90, TOV-81D, TOV-21D, OVCAR3, CAOV4, CAOV3, ES-2 or SKOV3. Hybridization to GAPDH and EEF1A-1 was used as a loading control.

Figures 3a through 3f show the oncogenic properties of EEF1A-2. In Figure 3a, NIH 3T3 lines were generated that express EEF1A-2. Protein expression of EEF1A-2 (with a V5 carboxy-terminal epitope tag) in independent EEF1A-2 -expressing lines (N1, N2, N3) is shown relative to parental and vector transfected controls. Figure 3b shows the diffuse cytoplasmic and non-nuclear localization of EEF1A-2 protein in transfected cells. Figure 3c shows that the EEF1A-2 -expressing lines grow as colonies in soft agar. The colonies shown are 15 days old. Figure 3d shows that the EEF1A-2 -expressing lines (N-1, N-2, N-3) all grow faster than parental NIH 3T3 cells. Figure 3e shows that EEF1A-2 transfection induces foci in Rat1 fibroblasts. Figure 3f shows a focus induced by EEF1A-2 having similar morphology to a focus induced by the RAS^{val12} allele.

Figure 4a through 4d show enhancement of tumorogenicity by EEF1A-2. Figure 4a shows that EEF1A-2-expressing NIH 3T3 cells grow as tumors in nude mice, while parental NIH 3T3 cells do not. Tumors shown are 21 days post injection. In Figure 4b, ovarian ES-2 lines were generated that express EEF1A-2. Protein expression of EEF1A-2 (with a V5 carboxy-terminal epitope tag) in independent lines (E1,E2,E3,E4) is shown relative to parental and vector transfected controls. In Figure 4c, the ES2 lines were injected into nude mice and tumor volume was measured as a function of time. EEF1A-2 – expressing cell lines grow faster than controls. The "sac." designation indicates that the animal was sacrificed due to ulceration of the primary tumor. Figure 4d shows the histology of ES-2-derived tumors (H&E 40X). Ischemic necrosis (n) and invasion of subcutaneous adipose (black arrows) and muscle (white arrows) tissues are indicated.

Figure 5 provides a line graph depicting the fractional survival, measured in days following diagnosis, plotted as a function of time for ovarian cancer patients with and without *EEF1A2* amplification. All tumors in these studies were stage III serous.

Detailed Description of the Invention

The genetic amplification of growth enhancing genes plays a key role in the development of human malignancy. Important to the understanding of oncogenesis is the identification of genes whose copy number and expression increases during tumorigenesis.

Agents that functionally inactivate these genes or proteins encoded thereby can be used as anticancer therapeutics. Furthermore, the genes and their protein products can be used as diagnostic and prognostic markers for disease progression and outcome prediction.

It has now been found that *EEF1A2*, the gene encoding EEF1A-2 (formerly eEF-1α2), is genetically amplified in 26% of primary ovarian and 25% of breast and colorectal tumors. In addition, as shown herein *EEF1A2* amplification correlates with significantly reduced survival among ovarian cancer patients. EEF1A-2 mRNA levels are also increased in 27% of primary ovarian tumors and 33% of established cell lines. The strong transforming and tumorigenic properties of *EEF1A2* are indicative of this gene and the protein encoded thereby having an important role in oncogenesis over and above any potential role as a 20q13 amplicon driver.

Further, it has now been found that EEF1A-2 has growth-promoting properties. Expression of EEF1A-2 alters the growth properties of mouse NIH 3T3 fibroblasts by increasing their growth rate and allowing them to grow in an anchorage-independent manner in soft agar. Expression of EEF1A-2 in RAT1 fibroblasts causes these cells to grow as a multi-layered focus. Anchorage-independent growth and focus formation are characteristics of cancerous cells. Importantly, expression of EEF1A-2 in NIH 3T3 cells makes these cells tumorigenic in mice. Expression of EEF1A-2 in the human ES2 ovarian carcinoma line increases the ability of these human cells to grow as tumors in nude mice. Thus, it is believed that EEF1A-2 is an oncogene, a gene that promotes cancer development.

To determine whether *EEF1A2* is part of the 20q13 amplicon in ovarian cancer, FISH (fluorescence *in situ* hybridization) was used to measure *EEF1A2* copy number in primary ovarian tumors. It was found that a 25% subset of primary ovarian tumors (14/53) have *EEF1A2* gene amplifications. Three representative primary ovarian tumor samples with amplifications are shown in Fig. 1a-c. Amplifications of *EEF1A2* are visualized by the increased number of loci hybridizing to an *EEF1A2* BAC (bacterial artificial chromosome) probe. The BAC probe contains the *EEF1A2* 3' UTR as determined by PCR. Hybridization of a control 20p11 probe to the same samples indicates that the increase in *EEF1A2*-hybridizing loci does not result from chromosome 20 polyploidy. A representative ovarian tumor with normal *EEF1A2* copy number is shown in Fig. 1d. As shown in the chromosomal metaphase spread in Fig. 1e, the BAC clone used

for the FISH hybridizes to 20q13. Thus, *EEF1A2* copy number is increased in a substantial subset of ovarian tumors and is part of the 20q13 amplicon.

To determine whether there is an increase in EEF1A-2 expression in ovarian tumors, Northern blotting was used to measure EEF1A-2 mRNA levels in primary ovarian tumors and established ovarian carcinoma cell lines. Although the tissue-specific expression pattern of human EEF1A-2 is currently unknown, rat and mouse EEF1A-2 RNA is expressed only in normal brain, heart and skeletal muscle (Lee et al. J. Biol. Chem. 1992 267:24064-24068; Knudsen et al. Eur. J. Biochem. 1993 215:549-554). As shown in Fig. 2a, EEF1A-2 message was undetectable in normal ovarian tissue (lane N), whereas 3/11 primary ovarian tumors (lanes 1,4,5) had readily detectable EEF1A-2 RNA. GAPDH and EEF1A-1 gene expression was similar among the samples. The EEF1A-1gene (*EEF1A1*) maps to 6q14, a locus not known to be involved in ovarian cancer. The presence of *EEF1A2* amplifications among the tumor samples is indicated in Fig. 2a. As shown herein, 2/3 tumor samples (lane 1 and 5) with elevated EEF1A-2 mRNA had increased *EEF1A2* copy number. One of the tumor samples with elevated EEF1A-2 expression did not have detectable *EEF1A2* amplification (lane 4), suggesting that EEF1A-2 expression may increase independently of *EEF1A2* copy number changes. EEF1A-2 mRNA expression is also increased in some established ovarian cancer cell lines relative to normal ovarian epithelial cells. Figure 2b shows that a normal ovarian epithelial cell line, NOV-61, has undetectable EEF1A-2 RNA. In contrast, 4/12 ovarian tumor cell lines (TOV112D, PA-1, HEY, 2008) expressed EEF1A-2. The OV-90, TOV81D, TOV21G, OVCAR3, OVCAR4, CAOV3, SKOV3 and ES-2 cells lines, like the normal NOV-61 cell line, did not detectably express EEF1A-2 mRNA. GAPDH and EEF1A-1 gene expression was similar among the cell lines. Taken together, Fig. 2a,b indicate that EEF1A-2 expression is increased in a ~30% subset of ovarian tumor samples and cell lines.

The oncogenic properties of human EEF1A-2 were also assessed. For these experiments, NIH 3T3 rodent fibroblast cell lines were established by stably expressing EEF1A-2 under the control of the CMV promoter. The EEF1A-2 used to generate the cell lines was tagged at its carboxy-terminus with the V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr (SEQ ID NO:1)) to facilitate detection by Western blotting. Protein expression of exogenous EEF1A-2 in three independent NIH 3T3 clones (N-1,N-2,N-3) is shown in Fig. 3a. As shown in Fig. 3b, the EEF1A-2 protein in

interphase cells is non-nuclear and diffusely cytoplasmic, corresponding to the wild type localization of the protein (Kjaer et al. Eur. J. Biochem. 2001 268:3407-3415). As shown in Fig. 3c, the EEF1A-2 -expressing clones grow as colonies in soft agar, a property not observed in the parental NIH 3T3 cells or NIH 3T3 cells transfected with the empty vector. Moreover, Fig. 3d shows that the EEF1A-2 -expressing clones have an accelerated growth rate relative to the parental NIH 3T3 controls. Four days after plating an equal number of cells, there were approximately four times as many EEF1A-2-expressing cells as parental cells, indicating that EEF1A-2 expression enhances cell growth rate.

The capacity of EEF1A-2 to enhance cell growth was also assessed by measuring the ability of EEF1A-2 to induce focus formation in Rat1 fibroblasts. The ability to form foci in cell culture is a marker for cell transformation and is considered one of the general properties of an oncogene such as *RAS* (Land et al. Nature 1983 Nature 304:596-602). As shown in Fig. 3e, *EEF1A2* induced focus formation in Rat1 cells. The constitutively active and transforming RAS^{val12} allele (Provencher et al. In Vitro Cell Dev. Biol. Anim. 2000 36:357-361) was used as a positive control. The morphology of *EEF1A2*-induced foci was similar to those induced by RAS^{val12} (Fig. 3f).

To determine whether EEF1A-2 enhanced tumorigenicity, EEF1A-2-expressing NIH 3T3 cells were subcutaneously injected into nude mice. As shown in Fig. 4a, expression of EEF1A-2 in NIH 3T3 cells was sufficient to induce *in vivo* tumorigenicity. No tumor growth was observed in the parental or vector-transfected NIH 3T3 cells. While the N-1 line expressed more EEF1A-2 protein than either N-2 or N-3, it did not appear to form larger tumors in the mice nor was it more efficient at forming colonies in soft agar (Fig. 3a). This indicates that N-1, N-2 and N-3 are expressing enough EEF1A-2 protein so that its abundance is not the limiting factor in either anchorage-independent growth or *in vivo* tumorigenesis.

To determine the effect of EEF1A-2 on an ovarian-derived cell, independent ES-2 ovarian cell lines that express EEF1A-2 (E-1,E-2,E-3,E-4) were generated. ES-2 are ovarian clear cell carcinoma cells that do not express detectable EEF1A-2 mRNA (Fig. 4a). Protein expression of EEF1A-2 in four independent ES-2 derivatives is shown in Fig. 4b. A non-specific background band of slightly higher molecular weight than the EEF1A-2 protein was seen in the parental and vector lanes and could also be discerned in the E-1, E-2, and E–3 lysates. As shown in Fig. 4c, the cell lines expressing EEF1A-2 all had an accelerated rate of tumor formation in nude mice relative to the ES-2 controls. Thus,

EEF1A-2 enhanced their *in vivo* tumorigenicity. Hematoxylin and Eosin stained sections of representative ES-2-derived tumors are shown in Fig. 4d. All tumors showed high-grade malignancy with an ischemic necrotic core (n) indicative of rapid tumorigenesis.

The demonstrated ability herein of EEF1A-2 promoting cancerous growth is indicative of EEF1A-2 being a target for anti-cancer therapy. It is believed that EEF1A-2 inactivation through inhibition of expression of EEF1A-2 and/or through inhibition of the activity of this protein will slow or stop the growth of cancer cells. Accordingly, one aspect of the present invention relates to methods of treating cancer by administering an agent that inhibits EEF1A-2 expression and/or activity. Such EEF1A-2 inactivating agents are expected to be particularly useful in the treatment of ovarian cancer. Other cancers, including breast and colorectal cancer, are also expected to be targets for EEF1A-2 inactivation.

In one embodiment, an anticancer agent comprises an antisense oligonucleotide which hybridizes to *EEF1A2* or mRNA thereof and inhibits transcription of EEF1A-2 and/or protein translation of EEF1A-2 mRNA. Antisense oligonucleotides can be delivered intravenously or orally in accordance with well known procedures. Alternatively, an adenovirus engineered to express an EEF1A-2-specific antisense oligonucleotide can be administered in accordance with routine procedures.

Other anticancer agents useful in the present invention may comprise small organic molecules, proteins, peptides or peptidomimetics that are capable of inactivating or inhibiting EEF1A-2.

The present invention also relates to screening assays for use in identifying potential anticancer agents based upon their ability to inactivate or inhibit EEF1A-2. For example, a screening assay of the present invention may comprise individually testing potential anticancer agents for their ability to inhibit: a) EEF1A-2-mediated enhancement of NIH 3T3 cell growth; b) EEF1A-2-mediated enhancement of protein translation; and/or c) EEF1A-2-mediated microtubule cleavage. The ability of a test agent to inhibit one or more of these activities is indicative of the agent being useful in the treatment of cancer, particularly ovarian, breast or colorectal cancer.

Another aspect of the present invention relates to the use of EEF1A-2 gene amplification or EEF1A-2 protein expression as a prognostic marker in cancer, particularly ovarian cancer, as well as breast and colorectal. As shown in Figure 5, ovarian cancer patients with *EEF1A2* amplification survived a shorter period of time

following diagnosis than ovarian cancer patients without *EE1FA2* amplification. Thus, detection of EE1FA2 amplification or increase EE1FA-2 protein expression can be used to prognosticate survival time of a cancer patient. In one embodiment, the presence of *EEF1A2* amplifications in primary ovarian tumors is identified using an *EEF1A2*-containing bacterial artificial chromosome (BAC). The presence of *EEF1A2* amplifications can be used as a genetic marker to predict the probability of survival. In another embodiment EEF1A-2 protein expression can serve as the prognostic marker of ovarian, breast or colorectal cancer. For example, the EEF1A-2 protein is not expressed in normal ovarian epithelial cells. Thus, antibodies that specifically recognize EEF1A-2 protein can be generated and used to stain samples of tumor removed from ovarian cancer patients. Patients with tumor samples that stain positive for EE1FA-2 are expected to survive for a shorter period of time as compared to patients with tumor sample negative for EEF1A-2. Prognostic information relating to *EEF1A2*-gene amplification and/or EEF1A-2 protein expression can be used to enhance clinical decision-making and to select appropriate treatment regimes.

In addition, increased EEF1A-2 expression in tumors such as primary ovarian tumors in a subject is expected to lead to increased EEF1A-2 protein levels in biological samples such as blood and other tissues obtained from the subject. Accordingly, measurement of increased EEF1A-2 levels in a biological sample such as plasma, serum or other tissue obtained from a subject can be used as a diagnostic tool for cancers such as ovarian, breast and colorectal cancer in the subject. Blood or other tissue samples can be taken from a subject and analyzed for the presence of EEF1A-2 protein using a standard immunoassay technique such as an ELISA with an EEF1A-2-specific antibody. Measured levels of EEF1A-2 protein in the sample can then be compared to levels in a control. As used herein, by "control" it is meant, a sample obtained from an individual known not have cancer, a sample obtained previously from the subject prior to the onset or suspicion of cancer, or a standard from data obtained from a data bank corresponding to currently accepted normal levels of this gene or gene product. Increased EEF1A-2 protein levels in the sample obtained from the subject as compared to levels in the control are indicative of the subject having ovarian, breast or colorectal cancer. The comparison performed may be a straight-forward comparison, such as a ratio, or it may involve weighting of one or more of the measures relative to, for example, their importance to the particular situation under

consideration. The comparison may also involve subjecting the measurement data to any appropriate statistical analysis.

Another aspect of the present invention relates to kits for diagnosing and prognosticating cancer in a subject by detecting *EEF1A2* gene amplification or EEF1A2 protein expression. Kits for detection of *EEF1A2* gene amplification preferably comprise a means for detection such as a *EEF1A2*-containing bacterial artificial chromosome (BAC) as well as instructions for use of BAC in detecting EEF1A2 gene amplification in tumor tissue samples. Kits for detection of EEF1A-2 protein expression preferably comprise a means for detection such as an antibody specific for EEF1A-2 as well as instructions for use of such antibody to immunoassay a biological sample such as a tumor tissue biopsy sample, or a serum or blood sample obtained from a subject for the presence of EEF1A-2. Other components included in these kits may comprise EEF1A2 standards, diluting solutions, and/or wash buffers routinely provided in diagnostic and prognostic kits of this nature.

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Fluorescence Hybridization and Microscopy

Fluorescence in situ hybridization (FISH) was performed in accordance with the procedure described by Demetrick, D.J. (Mod. Pathol. 1996 9:133-136). In these experiments, *EEF1A2* and 20p11 BAC clones were labeled with FITC-dUTP and Digoxygenin(DIG)-dUTP, respectively. The labeled clones were then hybridized at 37°C to interphase nuclei from frozen ovarian carcinoma tissue samples. Slides were counter-stained with DAPI and visualized utilizing a Zeiss Axioplan 2 microscope. A Photometrics PXL 1400 CCD camera was used to capture images of representative interphase nuclei and Electronic Photography version 1.3 Biological Detection software used for alignment. Adobe PhotoShop was used to pseudocolor FITC and DIG labeled probes. A V5 antibody (InVitrogen) diluted 1:500 in phosphate buffered saline (PBS) followed by an Alexa 546-conjugated (1:200 in PBS) secondary antibody was used to determine EEF1A-2 localization.

Example 2: RNA purification and Northern Blotting

Ovarian tumor samples were obtained from the Gynecology and Oncology Group of the Cooperative Human Tissue Network. RNA was prepared from 100-200 mg of frozen tumor homogenized in 2 ml of TriZol (Gibco) as per the manufacturer's directions. RNA from cell lines was obtained through lysis of a 60 mm plate with 1 ml of Trizol (Gibco). 10 ug of total RNA was loaded per lane and RNA was transferred to GeneScreen. Normal ovary mRNA was obtained from Stratagene. Membranes were pre-hybridized at 63°C in 25 ml Church's Buffer, hybridized in 15 ml Church's at 59°C overnight, and washed at 62°C. The EEF1A-2 probe was a 598 *BamHI/PstI* fragment of the human *EEF1A2* cDNA.

Example 3: Cell culture and Western blotting

NIH 3T3 and ES-2 cells were grown in 10% FBS/DMEM and 10%FBS/McCoy's 5A respectively. EEF1A-2-expressing NIH 3T3 and ES-2 cells were derived by transfecting NIH 3T3 cells with 5 ug of the EEF1A-2 plasmid and 15 ul of SuperFect (Qiagen) per 60 mm dish. 0.4 mg/ml Zeocin (InVitrogen) was used to select transfectants and independent clones derived by limiting dilution cloning. An α-V5 antibody (InVitrogen; 1:500 in TBST) followed by an HRP conjugated goat anti-mouse (BioRad; 1:1,000 in TBST) and ECL+ (Amersham) were used to detect EEF1A-2 expression. Cell growth was measured by Coulter counting triplicate independent platings from a NUNC 6-well plate. For focus-forming assays, *EEF1A2* and RAS^{val12}, both under the control of the CMV promoter, were transfected into Rat1 fibroblasts using calcium phosphate according to the manufacturer's directions (Gibco). The pCDNA3 empty vector was used as a control. Transfected cells were grown in 2% FBS/DMEM at 37°C for 14 days and the media changed every three days. Transfection efficiency was determined by counting colonies that arise in selective media (Zeocin for EEF1A-2 and G418 for Ras). Foci were counted by washing plates in PBS, fixing in 10% acetic acid and staining with 0.4% crystal violet. Counts are the mean of triplicate experiments, each containing triplicate independent transfections. For soft agarose assays, 2×10^4 NIH 3T3 cells were placed in 3 ml of 0.35% low gelling temperature agarose (Sigma) in 10% FBS/DMEM and overlaid on 5 ml 0.8% agarose/10% FBS/DMEM in a 60 mm dish. Cells were grown at 37 °C for 14 days to allow colony formation.

Example 4: Tumor Xenografts

NIH 3T3 or ES-2 cells (1×10^6) were injected subcutaneously into the hind leg of nude mice and the animals were sacrificed 21 days post injection. Tumor volume (V) was estimated from the length (l) and width (w) of the tumor by the formula: $V = (\pi/6) \times ((l + w)/2)^3$. Tumors were fixed in formalin overnight at 4^0C and paraffin embedded. Sections were de-waxed and stained with Haematoxylin and Eosin. Animal experiments were conducted through protocols approved by the Central Animal Facility at McMaster University.

What is Claimed is:

1. A method for diagnosing cancer in a subject comprising measuring *EEF1A2* gene amplification or EEF1A-2 protein expression levels in a biological sample obtained from the subject and comparing the measured *EEF1A2* gene amplification or EEF1A-2 protein expression levels with levels of *EEF1A2* gene amplification or EEF1A-2 protein expression levels in a control wherein an increase in the measured *EEF1A2* gene amplification or EEF1A-2 protein expression levels as compared to the control is indicative of the subject having cancer.

2. The method of claim 1 wherein the cancer is ovarian cancer, breast cancer or colorectal cancer.

3. The method of claim 1 wherein the biological sample comprises a serum or plasma sample or a tumor tissue biopsy sample obtained from the subject.

4. A method for prognosticating survival and selecting effective treatment regimes for a patient suffering from cancer comprising measuring *EEF1A2* gene amplification or EEF1A-2 protein expression levels in a biological sample obtained from the subject.

5. The method of claim 4 wherein the patient is suffering from ovarian, breast or colorectal cancer.

6. The method of claim 4 wherein the biological sample comprises a serum or plasma sample or a tumor tissue biopsy sample obtained from the patient.

7. A kit for prognosticating and/or diagnosing cancer comprising a means for measuring *EEF1A2* gene amplification or EEF1A-2 protein expression levels in a biological sample.

8. A method for treating cancer comprising administering to a patient suffering from cancer an inhibitor of EEF1A-2 expression and/or activity.

9. A screening assay to identify new anticancer agents comprising measuring an agent's ability to inhibit EEF1A-2 expression and/or activity.

10. The screening assay of claim 9 wherein the agent's ability to inhibit EEF1A-2 expression and/or activity is measured by EEF1A-2-mediated enhancement of NIH 3T3 cell growth, EEF1A-2-mediated enhancement of protein translation or EEF1A-2-mediated microtubule cleavage.

ABSTRACT

Methods and kits for diagnosing and prognosticating cancer via detection of *EEF1A2* and/or EEF1A-2 are provided. Also provided are methods for treating cancer via inhibition of expression and/or activity of EEF1A-2 and screening assays to identify new anticancer agents based upon their ability to inhibit EEF1A-2 expression and/or activity. The methods and kits of the present invention are particularly useful in the prognosis, diagnosis and treatment of ovarian, breast and colorectal cancer.

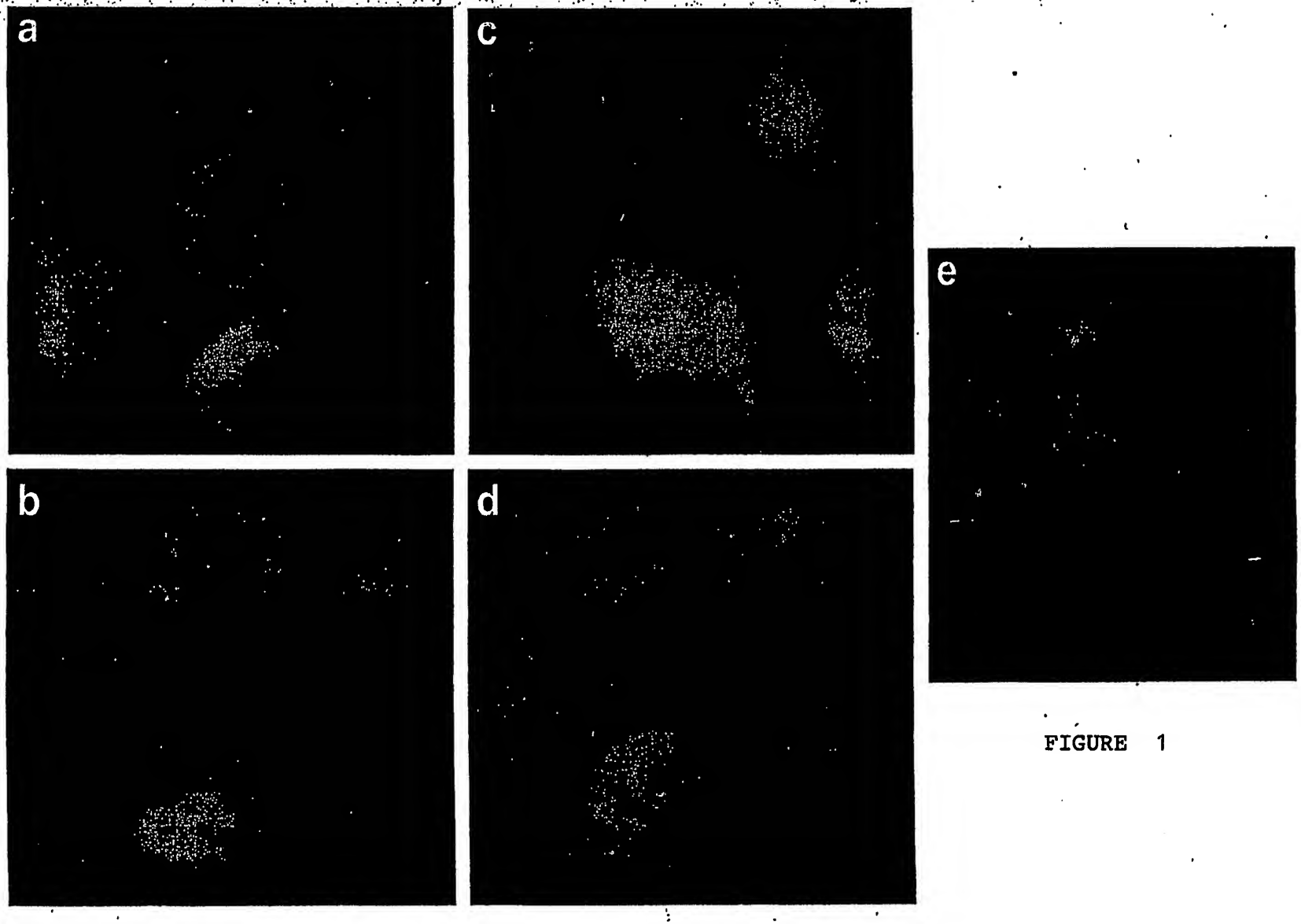
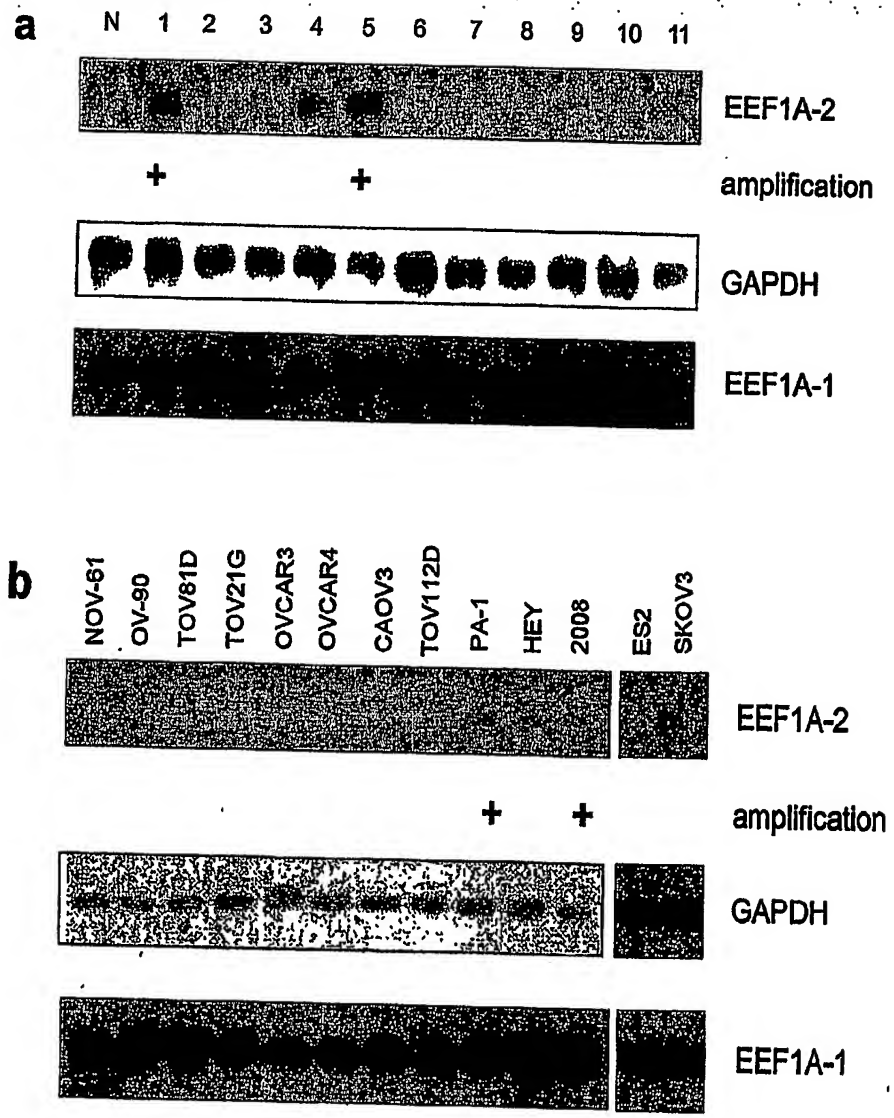


FIGURE 1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EEF1A-1
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TOV21G
OVCAR3
OVCAR4
CAOV3
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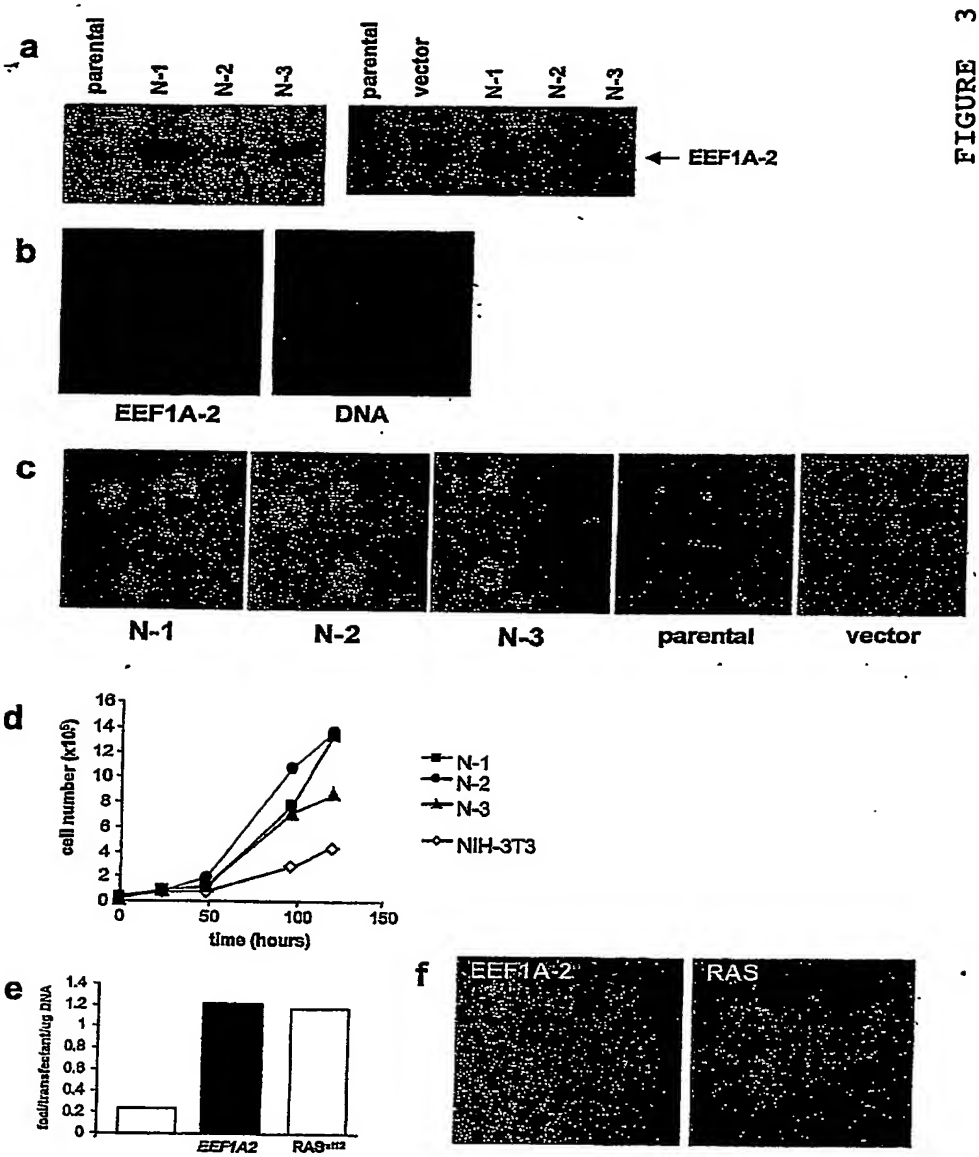
amplification
GAPDH
EEF1A-1

FIGURE 2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DNA
c
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vector
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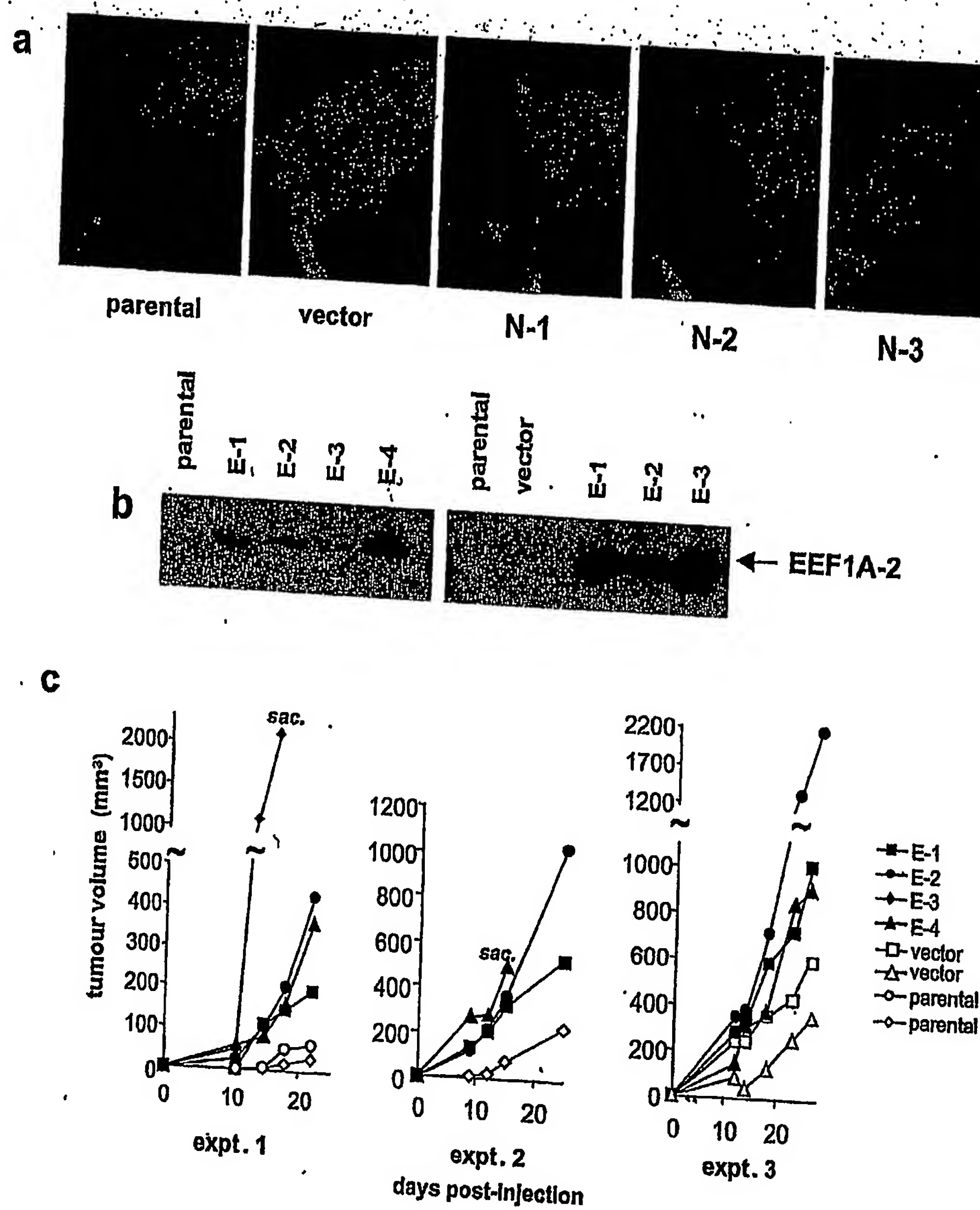
RAS

FIGURE 3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sac.
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FIGURE 4

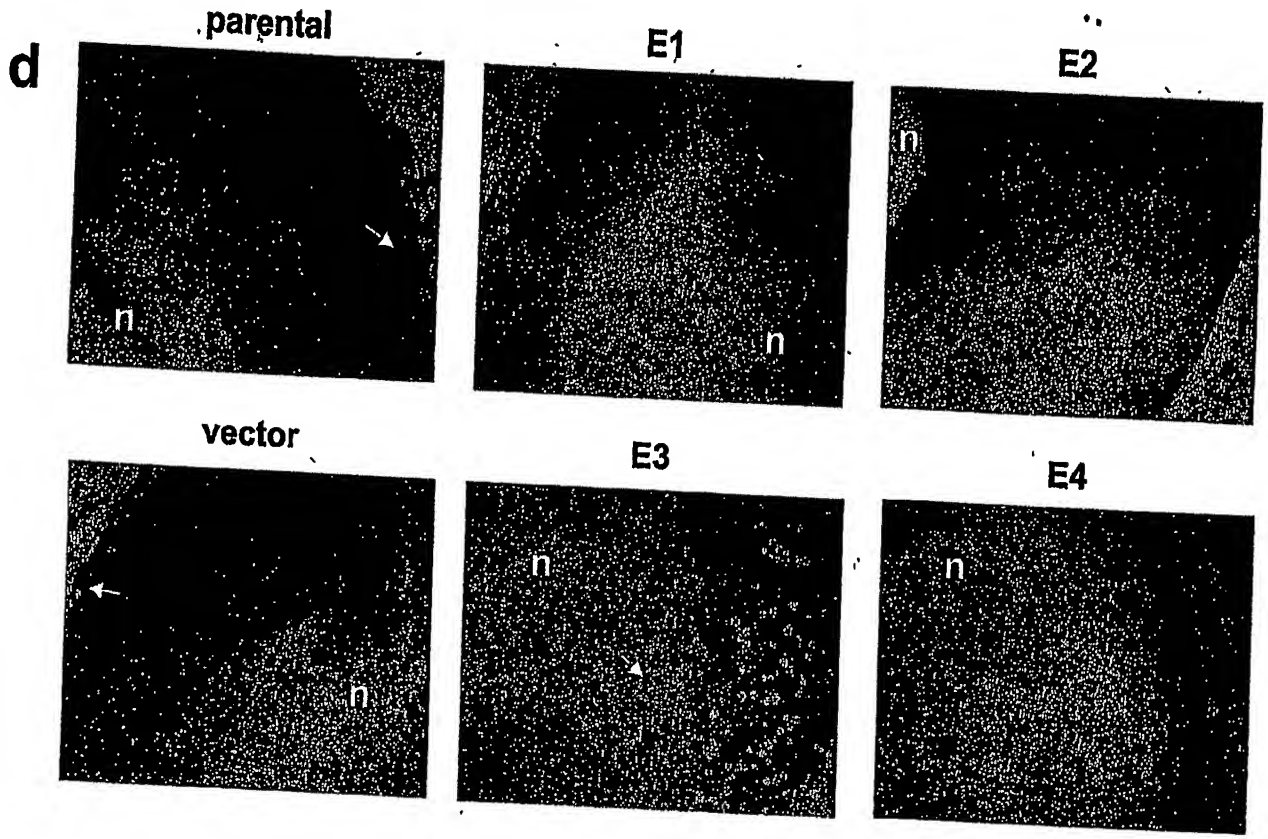

FIGURE 4 (cont'd)

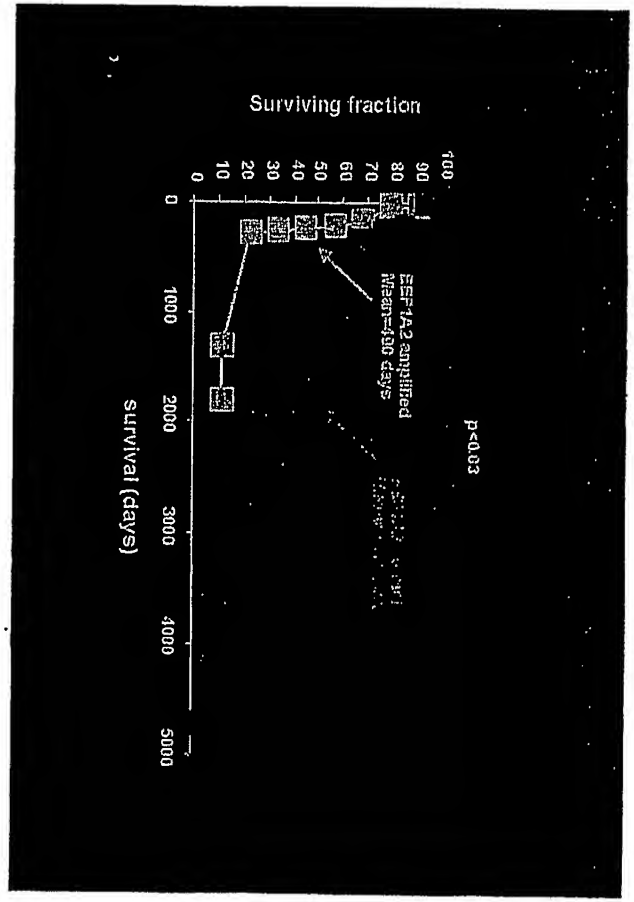

FIGURE 5